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Effects of antidromic stimulation of the ventral root on glucose utilization in the ventral horn of the spinal cord in the rat

(cell bodies/axon terminals/2-deoxy[^{14}C]glucose)

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ABSTRACT **Electrical stimulation of the proximal stump of the transected sciatic nerve increased glucose utilization in the ventral horn of the spinal cord, with the greater increase in Rexed's lamina IX. Antidromic stimulation of the ventral root, however, did not change glucose utilization in the ventral horn. These results suggest that the axon terminals and not the cell bodies are the sites of enhanced metabolic activity during increased electrical activity in these elements.**

The deoxyglucose method has been widely used to map functional neural pathways on the basis of evoked metabolic responses. When neurons are electrically active, the intracellular concentration of Na^+ and extracellular concentrations of K^+ increase, stimulating the activity of the Na^+,K^+-ATPase, with consequent enhancement of glucose utilization (1). The metabolic responses are positively correlated with the frequency of spikes (2). It has not been clearly established, however, whether this increased glucose utilization occurs throughout the neuron or is localized to particular cell elements (e.g., perikaryon, dendrites, initial segment, or axon terminals). Evidence from studies in vertebrates indicates that areas rich in neuropil are metabolically more activated than areas rich in cell bodies. For example, functional stimulation of the hypothalamus-neurohypophysial system in rats by salt-loading increases glucose utilization in the terminals of this pathway in the pituitary neural lobe but not in the paraventricular and supraoptic nuclei, where the cell bodies of origin of the tract are located (3). *In vitro* studies in the molluscan nervous system, however, indicated that the perikaryon can increase the deoxy[^{3}H]glucose uptake in response to increased electrical activity (4, 5).

The objective of the present experiments was to examine with the quantitative autoradiographic 2-deoxyglucose method (6) whether cell bodies increase their rates of glucose utilization in response to increased spike activity. Two experimental models were used. In the first model, the sciatic nerve was electrically stimulated, and in the second the ventral root of the spinal cord was antidromically stimulated. In both cases glucose utilization was measured in the ventral horn of the spinal cord. In a previous study (7) the effects on glucose utilization in the dorsal root ganglia and dorsal horn of the spinal cord in response to electrical stimulation of the sciatic nerve were examined; the results showed that the axon terminals in the dorsal horn, and not the cell bodies in the dorsal root ganglia, are the sites of increased metabolic activity during stimulation of the sciatic nerve. In the present report, we examine the effects of electrical stimulation of the sciatic nerve on glucose utilization in the ventrolateral Rexed's lamina IX, where the motoneurons are located, and in the ventromedial zone, laminae VII and VIII (8), where the collaterals of the motor axons synapse with interneurons (9). Electrical stimulation of the sciatic nerve caused a slight frequency-independent increase in glucose utilization in Rexed's lamina IX, an area rich in motoneurons, and in laminae VII and VIII, areas rich in interneurons. Antidromic stimulation of the ventral root did not, however, increase glucose utilization in laminae VII and VIII or IX. These results provide further evidence that cell bodies are not the sites of enhanced metabolic activity during increased spike activity.

MATERIALS AND METHODS

Chemicals. 2-Deoxy-D-[1-^{14}C]glucose (specific activity = 50–55 mCi/mmol; 1 Ci = 3.7×10^{10} Bq) was purchased from New England Nuclear. Calibrated [^{14}C]toluene, used for internal standardization in the measurement of plasma deoxy[^{14}C]glucose concentrations, was also obtained from New England Nuclear. Atropine sulfate was purchased from Invenex (Chagren Falls, OH) and tubocurare was from Abbott.

Animals. Adult male Sprague–Dawley rats (300–400 g) were purchased from Taconic Farms (Germantown, NY) and Harlan Sprague Dawley (Indianapolis, IN). Prior to the experiment, the animals were allowed water and Purina Laboratory Chow ad libitum and were kept in a controlled environment with alternate 12-hr light and dark cycles (6:00 a.m.–6:00 p.m., light).

Preparation of the Animals. Two experimental groups were prepared. In the first group the animals (n = 12) were anesthetized by an intraperitoneal injection of 45 mg of sodium pentobarbital per kg of body weight, and polyethylene catheters were inserted in one femoral artery and vein. The sciatic nerves on both sides were exposed, tied, and transected bilaterally at the level of the gluteus muscles. A wire loop was inserted around the skin incision on each side and drawn to make pools in which paraffin oil, used to prevent desiccation of the nerves, could be retained.

In the second group the animals (n = 8) were anesthetized by an intraperitoneal injection of 1.2 g of urethane per kg of body weight. Polyethylene catheters were inserted in one femoral artery and vein. A dose of 100 μg of atropine sulfate was administered intravenously to prevent mucous secretion in the respiratory tract. The trachea was exposed and isolated and a cannula was inserted in it. The animals then were paralyzed by an intravenous injection of 300 μg of tubocurare and artificially ventilated with 100% oxygen. A laminectomy was performed at the L_3–L_6 level. Special care was taken not to compress the spinal cord. The dura matter was incised, and

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the dorsal and ventral roots were identified and sectioned at L_5 on both sides. The spinal cord and the roots were maintained under paraffin oil to prevent desiccation.

Electrical Stimulation. In the first group, the proximal portion of one transected sciatic nerve was placed on bipolar platinum electrodes and stimulated by means of a stimulus isolation unit with pulses 0.2 ms in duration at a current intensity of 200–400 μA and at a frequency of 5 Hz (n = 4), 10 Hz (n = 4), or 15 Hz (n = 4). The current intensity was continuously monitored on an oscilloscope. The stimulating cathode was positioned proximal to the spinal cord. The effectiveness of the electrical stimulation was monitored by observation of the reflex contraction of the gluteus muscles.

In the second group the proximal portion of one transected ventral root was placed across two pairs of silver electrodes. The distal pair was used to stimulate the root, with the cathode positioned proximal to the spinal cord, and the proximal pair was used to record the compound action potential, which was continuously monitored on an oscilloscope. By recording the compound action potential, the viability of the root could be assessed and the conduction velocity of the fibers could be calculated. The root was stimulated with pulses 0.2 ms in duration at a frequency of 50 Hz (n = 2), 60 Hz (n = 2), 70 Hz (n = 2), or 90 Hz (n = 2) and at a current intensity 1.5 times the current that produced the maximum amplitude in the compound action potential (48–96 μA). In two animals the dorsal root was stimulated with pulses 0.2 ms in duration at a frequency of 60 Hz and intensity of 60 μA to check the responsivity of the preparation.

Electrical stimulation in all preparations was begun 5 min before the initiation of the period of measurement of glucose utilization and was continued until ≈5 min before the termination of the experimental period.

Measurement of Glucose Utilization. The period of measurement of glucose utilization was initiated by the intravenous administration of a 125 μCi/kg pulse of 2-deoxy[1-^{14}C]-glucose. Timed arterial blood samples were drawn throughout the following 45 min, and the plasma was assayed for deoxy[^{14}C]glucose and glucose concentrations as described (6). At ≈45 min after the pulse of deoxy[^{14}C]glucose the animals were killed by an intravenous injection of sodium pentobarbital. The lumbar spinal cord was removed, frozen in isopentane chilled to −45°C with dry ice, and covered with embedding medium (M-1 embedding matrix, Lipshaw Manufacturing, Detroit, MI). The lumbar cords were stored at −70°C until sectioned (20 μm) and autoradiographed as described (6). Selected sections were stained with thionin for histological identification of areas of interest and for examination for injury. The local tissue concentrations of ^{14}C were determined by quantitative densitometric analysis of the autoradiographs of the spinal cord and the calibrated standards by means of (*i*) a Photoscan P-1000 microdensitometer (Optronics International, Chelmsford, MA) and the image-processing system described by Goochee *et al.* (10) and (*ii*) a moving linear array charge-coupled-device camera (Scientific Imaging, Santa Clara, CA) and an image-processing system described by Lear *et al.* (11). Glucose utilization was calculated from the tissue concentration of ^{14}C and the time courses of the arterial plasma deoxy[^{14}C]glucose and glucose concentrations by means of the operational equation of the deoxyglucose method (6).

Physiological Status. The physiological status of the animals was assessed during the experimental period by monitoring of mean arterial blood pressure, arterial blood pH, P_{O_2}, P_{CO_2}, hematocrit, and plasma glucose concentration. Body temperature was maintained between 36°C and 37°C by means of an electrical heating pad.

Statistics. Data for each of the stimulation studies were statistically analyzed by a split-plot analysis of variance, which, for this study, is a type of paired t test. For these analyses the denominator of the t statistic was formed by averaging the variances calculated within each of the stimulation frequency groups rather than by using a single overall variance that ignores the frequency groups as is done with the more familiar paired t test. Pooling the variances in this way adjusts the denominator of the t statistics for any systematic effects of stimulation frequency that may be present in the data. The primary statistical tests assessed the effect of electrical stimulation within each lamina.

RESULTS

Physiological Variables. The physiological variables in the sodium pentobarbital-anesthetized group of rats have been published (7). Briefly, the arterial blood pH and P_{O_2} were slightly below and P_{CO_2} was slightly higher than the values observed in normal conscious rats.

The physiological data for urethane-anesthetized rats are summarized in Table 1. Urethane is known to cause a drop in arterial blood pressure and to increase plasma glucose concentration and hematocrit when compared to values in conscious animals (12). Arterial blood pH remained within the normal physiological range. Due to artificial ventilation with 100% O_2, arterial P_{O_2} was higher and arterial P_{CO_2} was lower than values in conscious animals breathing spontaneously (12).

Effects of Electrical Stimulation of Sciatic Nerve on Glucose Utilization in the Ventral Horn of the Spinal Cord. Electrical stimulation of the sciatic nerve resulted in slightly increased glucose utilization in the ventral horn of the spinal cord, with the greatest increases in Rexed's lamina IX (Table 2). The mean glucose utilization rates with the three stimulation frequencies were not significantly different, indicating no frequency dependency.

Effects of Antidromic Stimulation of the Ventral Root on Glucose Utilization in the Ventral Horn of the Spinal Cord. Antidromic stimulation of the ventral root of the spinal cord activated fibers with conduction velocities ranging between 20 and 100 m/s. The results summarized in Table 3 and Fig. 1 show that antidromic stimulation of the ventral root does not change glucose utilization in laminae VII and VIII or IX of the ventral horn of the spinal cord. Stimulation of the dorsal root, however, increased glucose utilization in the dorsal horn of the spinal cord (Fig. 1), demonstrating that glucose utilization is responsive to increased afferent input.

DISCUSSION

The results of the present experiments demonstrate that electrical stimulation of the sciatic nerve increases glucose utilization in Rexed's laminae VII and VIII and IX, particularly in lamina IX, where the motoneurons are located (8). Stimulation of the sciatic nerve activates nerve fibers orthodromically and antidromically. It appears, however, that the increase in glucose utilization observed in the ventral horn of the spinal cord is a reflection of the orthodromic activation of the axon terminals synapsing with motoneurons because

Table 1. Physiological variables in animals anesthetized with urethane

Mean arterial blood pressure (torr)	77 ± 7
Arterial blood	
pH	7.45 ± 0.02
P_{O_2} (torr)	159 ± 9
P_{CO_2} (torr)	29 ± 2
Hematocrit (%)	57 ± 3
Plasma glucose concentration (mg/ml)	3.04 ± 0.13

Values are means ± SEM, n = 8.

Table 2. Effects of unilateral sciatic nerve stimulation on glucose utilization in the ventral horn of the spinal cord

	Glucose utilization, μmol/100 g per min	
	Stimulated side	Control side
Rexed's laminae VII and VIII	46 ± 1*	43 ± 1
Rexed's lamina IX	46 ± 2*	41 ± 1

Values are means ± SEM, $n = 12$, of all animals, regardless of stimulation frequency. There were no significant differences among values at different frequencies of stimulation.
*Statistically significant difference compared with control unstimulated side; $P < 0.01$ by a split-plot analysis of variance.

antidromic stimulation of the ventral root of the spinal cord does not increase glucose utilization in Rexed's lamina IX. It is likely that the antidromic stimulation of the ventral root invaded the region of the cell bodies in the ventral horn because in all of the experiments the compound action potential could be recorded during the whole period of stimulation. That this kind of preparation is responsive to increased afferent input was shown by the enhanced metabolic activity in the dorsal horn of the spinal cord following electrical stimulation of the dorsal root. It seems, therefore, that the neuropil, and not the cell bodies, is the site of increased glucose utilization during increased electrical activity.

In a previous study (7) we have shown that electrical stimulation of the sciatic nerve induces a frequency-dependent increase in glucose utilization in the region of the afferent terminals in the dorsal horn of the spinal cord but not in the cell bodies of the dorsal root ganglion. Nudo and Masterton (13) observed that the active synapses, whether excitatory or inhibitory, are the sites of increased deoxyglucose uptake and not the discharging postsynaptic membrane. The increased metabolic activity during increased impulse activity is probably a reflection of the activity of the Na^+,K^+-ATPase needed to restore the resting ionic distribution across the membrane following the action potential. Mata *et al.* (14) have shown that stimulation of deoxy[^{14}C]glucose uptake in slices of pituitary neural lobe by electrical stimulation *in vitro* is blocked by ouabain, a specific inhibitor of the Na^+,K^+-ATPase.

Our results provide evidence that postsynaptic elements do not increase their glucose utilization in response to increased electrical activity. These observations are compatible with electrophysiological evidence obtained with the patch electrode (15) that the soma and dendrites of spinal cord neurons and the soma of dorsal root ganglion cells of mouse grown in tissue culture do not generate action potentials. Similar findings have been described in other systems (16, 17). By an analysis of extracellular potentials from single spinal motoneurons and single neurons in the lateral geniculate nucleus, Freygang (16) and Freygang and Frank (17) presented evidence that most of the soma-dendritic membrane can be excited synaptically to produce postsynaptic potentials but not propagating action potentials. This idea is in contrast to the more traditional concept that soma and dendrites do fire action potentials (18–23). If so, then the results of our experiments might be explained by a smaller surface-to-volume ratio in the cell bodies than in the axon terminals, which would lead to less energy expenditure to restore the resting membrane potential following an action potential at the perikarya than at the axon terminals (3). One observation that requires further investigation is that glucose utilization did not increase in laminae VII and VIII where collaterals of motor axons synapse with interneurons (8). It is known that this pathway is activated when motor axons are stimulated (24, 25). It may be that the density of these collaterals is too small to alter significantly the rates of glucose utilization by a magnitude detectable by the deoxyglucose method.

Table 3. Effects of antidromic stimulation of ventral root on glucose utilization in the ventral horn of the spinal cord

	Glucose utilization, μmol/100 g per min	
	Stimulated side	Control side
Rexed's laminae VII and VIII	18 ± 4	19 ± 4
Rexed's lamina IX	17 ± 3	17 ± 3

Values are means ± SEM, $n = 8$, of all animals, regardless of stimulation frequency. There were no frequency-dependent differences.

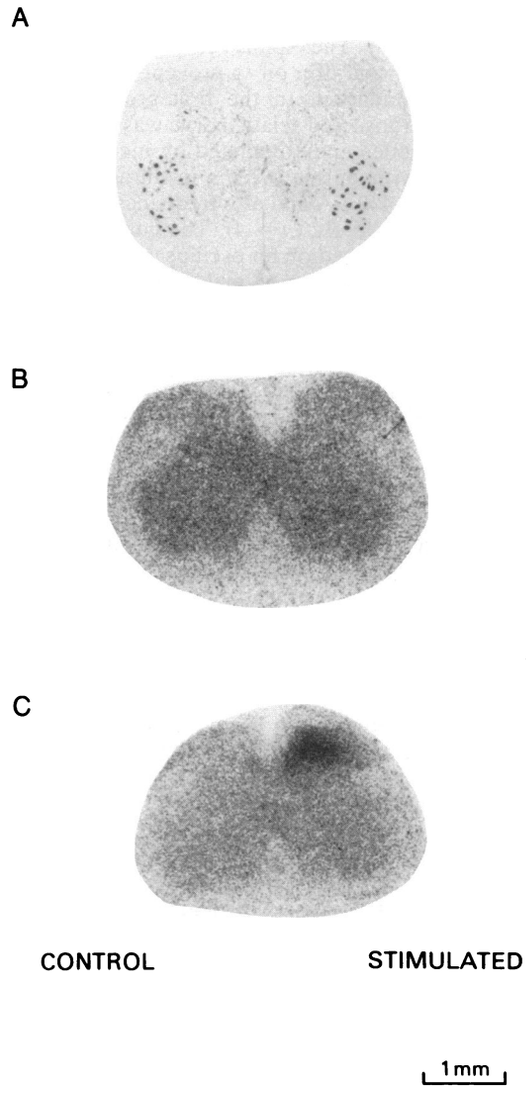

FIG. 1. Thionin-stained frontal section of the lumbar spinal cord (*A*) and deoxy[^{14}C]glucose autoradiographic images of the lumbar spinal cord sections during electrical stimulation of the ventral root (*B*) and of the dorsal root (*C*). The higher optical density of the dorsal horn on the stimulated side in *C* reflects an increased rate of glucose utilization during stimulation of the dorsal root. Note that antidromic stimulation of the ventral root does not increase glucose utilization in the ventral horn (*B*).

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